Regeneration of a New Hair Follicle from the Upper Half of a Human Hair Follicle in a Nude Mouse

To the Editor

The hair follicle (HF) represents a self-renewing system and growing evidence indicates that a whole HF can be regenerated from portions of the HF. After the follicular bulbs were removed, dermal papilla and then bulb structures have been shown to be reformed, and hair fiber growth restored (Oliver, 1967; Jahoda *et al*, 1996; Hashimoto *et al* 2001). Similarly, Kim *et al* (1996) have described regeneration of human HF from transversely sectioned HF after autologous grafting. The new dermal papilla has been shown to be regenerated from the remaining connective tissue sheath (Jahoda *et al*, 1996; Kim *et al*, 1996). Furthermore, the regenerated HF from the lower half implant showed that the sebaceous gland was completely reformed (Kim *et al*, 1996). The athymic nude mouse has been a reasonable model for studying human hair transplantation, as well as HF physiology and pathology (Van Neste, 1996; Van Neste and De Brouwer 2000); however, the regenerative capacity of different fragments of the human HF has not been tested on nude mice. In this study, we have evaluated the regenerative capacity of transversely sectioned human HF in athymic nude mice.

The HF were obtained from the occipital areas of patients undergoing hair transplantation. The individual HF was cut into different segments under a dissection microscope. Three groups of transected HF were used for grafting on to nude mice. One group was sectioned at the bulge underneath sebaceous gland into two halves. The second group was equally tri-sectioned at the middle of sebaceous gland and below the bulge, respectively. The last group was cut into four segments with the same length for each HF. First, the HF was sectioned into two halves as the first group. The upper half was then sectioned at the entrance of the sebaceous gland duct into the uppermost portion (the infundibulum) and the second upper segment (mainly the isthmus). The lower half was cut equally into another two segments with the lowermost portion mainly containing the hair bulb. All the HF portions were maintained in saline solution before being transplanted on to nude mice. A total of 12 HF was transplanted for each group.

Mice were purchased from Harlan Sprague Dawley (Indianapolis, IN). Recipient mice were anesthetized with 20 mg ketamine and 5 mg xylazine per kg body weight administrated intraperitoneally. Sites on the dorsal skin of each mouse were tattooed for each group/subgroup of follicles. A small incision was made and the portions of the follicle were transplanted underneath the mouse skin in each tattooed area. At the end of the operation, a strip of Op-Site (Johnson & Johnson, New Brunswick, NJ) was applied to seal the incisions. Grafts were observed for survival and growth at 1 wk intervals for 4 mo postoperatively. At the end of the experiment, the dorsal back of each mouse was skinned off and the growth of the transplanted HF was examined macroscopically. The areas with implanted human HF were biopsied and processed for histology analysis.

Among all the transplanted HF fragments, one upper portion of a bisected human HF was able to regenerate a completely new, pigmented hair of 10 mm long underneath the nude mouse skin after 16 wk of transplantation. The new hair fiber was thinner than a normal human anagen hair (data not shown). The original HF was transected below the sebaceous gland before grafting. The regenerated HF was about 2.5 times smaller than the original one in diameter (**Fig 1**). Histologic analysis revealed the presence of a completely reformed follicle containing all the follicular structures, including dermal papilla, outer root sheath, inner root sheath, and pigmented hair cortex (**Fig 1*a,c***). The new dermal papilla and sebaceous gland were obviously regenerated from the dermal sheath cells and outer root sheath cells of the original HF, respectively (**Fig 1*b,d***).

In this pilot study, the rate of graft take and survival was very low. Only one upper half of the human HF implant was able to regenerate a complete new HF. In all other HF implants no visible hair fiber was reproduced within the mouse skin and no follicular structures were observed histopathologically, indicating that the implanted portions of HF were not able to survive throughout the

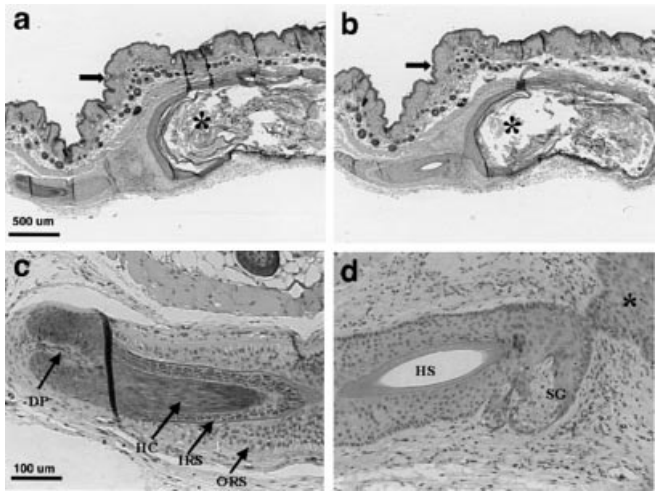

Figure 1. Histologic features of a new human HF regenerated underneath the nude mouse skin. The upper half of a human HF was implanted on to the nude mouse skin and biopsied after 16 wk. (*a,b*) The small new HF (left) was regenerated from the originally transplanted upper half of the follicle (*). The human HF is located underneath the mouse skin (*arrow*) in parallel to the mouse skin surface. The new HF was sectioned at different positions in *a* and *b*. (*c*)Higher magnification of the lower portion of the new HF from *a*.All elements of the newly formed follicular structures, including dermal papilla (DP), outer root sheath (ORS), inner root sheath (IRS), and pigmented hair cortex (HC), are present. (*d*)Higher magnification of the new HF sectioned near the end of the original HF (*) from *b*.New hair shaft (HS) was formed in the middle and the sebaceous gland tissue (SG) was outgrowing. The specimen was stained with hematoxylin and eosin. Scale bars: (a,b) 500 μm; (c,d) 100 μM.

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experiment. This low successful regeneration rate may be attributed to the implantation technique in which transected human HF were placed on the incised skin surface. By transplanting the HF under panniculus carnosus, Kurata *et al* (1999) improved the hair growth (hair fiber elongation) rate to over 60%. Although we were not examining hair fiber elongation in this study, the successful rate may be improved if the portions of HF are implanted beneath the incised mouse skin at the level of panniculus carnosus.

To the best of our knowledge this is the first study showing that a completely new HF can be regenerated from the upper half portion of human HF on a nude mouse. It is generally agreed that in hair transplantation HF transplanted on to a recipient site undergo catagen and telogen, and new anagen hairs are grown. For fragments without hair bulbs, a new dermal papilla needs to be regenerated from the connective sheath cells and then a new follicle will be reformed through interactions between dermal papillae and follicular keratinocytes. The upper portion of the HF contains the population of stem cells and some connective sheath cells (Reynolds *et al*, 1993; Rochat *et al*, 1994; Akiyama *et al*, 1995; Moll, 1995), from which a new sebaceous gland, the epithelial parts of the new HF, and the new dermal papilla may have been reformed, respectively. If the success rate of this graft technique can be improved, this study could address the question of what are the minimum requirements for regenerating a new human HF from a transected HF. Eventually, the smallest ''follicular forming unit'' can be defined.

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